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Your reference	REP05320GB
Patent application number *(The Patent Office will fill in this part)*	29 NOV 1996 **9624927.1**
Full name, address and postcode of the or of each applicant *(underline all surnames)*	Oxford GlycoSciences (UK) Limited Hitching Court Blacklands Way Abingdon OX14 1RG United Kingdom
Patents ADP number *(if you know it)*	
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
Title of the invention	GELS AND THEIR USE
Name of your agent *(if you have one)*	GILL JENNINGS & EVERY
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Broadgate House 7 Eldon Street London EC2M 7LH
Patents ADP number *(if you know it)*	745002

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Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	YES

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Description	9
Claim(s)	2
Abstract	
Drawing(s)	1

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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date
29 November 1996

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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GELS AND THEIR USE

Field of the Invention

This invention relates to gels, and particularly to gels suitable for use in the separation, by electrophoresis, of species in a biological sample.

Background of the Invention

Proteins and glycoproteins are the central effectors of health and disease at the cellular level. Numerous attempts are being made to use molecular genetics and genomics to discover proteins and glycoproteins central to disease and infectious processes, as potential molecular targets for drug delivery, as therapeutic entities in their own right and as clinical diagnostic and prognostic markers. While genomics has been a major advance in understanding the molecular basis of biological processes, it has currently two significant limitations.

Firstly, direct information on post-translational modifications of a protein cannot be deduced from a knowledge of its gene sequence. It is now apparent that a large proportion of gene products exist in their final state in a post-translationally modified form. Furthermore, post-translational modifications (such as glycosylation and phosphorylation) can profoundly influence and control the biochemical properties of a protein. Understanding of the structure and function of a protein, or of a biochemical/disease process, is often incomplete without a knowledge of the dynamic control of post-translational modifications. Secondly, there are increasing instances of post-translational control of protein expression, i.e. the presence or absence of mRNA does not correlate to the presence or absence of the encoded protein.

For these reasons, a next step forward is to build on genomic data by understanding the patterns of protein and carbohydrate expression, and of post-translational modification generally in a biological or disease process

through direct analysis of expressed proteins and glycoproteins.

In the general case, glycoproteins and proteins are presented as complex mixture. Therefore, separation into individual components is a necessary step in their analysis. For example, the electrophoretic separation of species, e.g. proteins or carbohydrates, and including glycoproteins, is a well known technique. It is also known to use labelling techniques, e.g. with a fluorescent label, for detection of the species.

Gel electrophoresis of proteinaceous material is usually performed using free gels or gels backed onto flexible plastics material. Neither form is suitable for both spectroscopic (and preferably, fluorescence-based) detection of proteins in gels and automated excision of protein spots. In the case of free gels, the lack of a support makes automated excision of spots inaccurate and unreliable; the gel is not sufficiently mechanically stable to allow accurate maintenance of its position on a cutting platform. In the case of plastics-backed gels, the nature of plastics materials, which are highly spectrally-active, provides a background, and particularly a fluorescent one, which precludes detection of spectrally/fluorescently-active proteins in the gel.

Summary of the Invention

This invention is based on appreciation of the fact that the disadvantages described above can be overcome, with consequential important, practical advantages. In particular, this invention provides an automated process for high-throughput parallel analysis of proteins and their post-translational modifications, and also a protein-based approach to functional genomics.

A first aspect of the present invention is a supported two-dimensional gel suitable for use in electrophoresis, in which the gel is covalently bonded to a solid support such that the gel has two-dimensional spatial stability, and the

support is substantially non-interfering with respect to detection of a detectable label bound to the species.

The spatial integrity of such a gel allows portions thereof, containing separated species of interest, to be removed, e.g. excised, for analysis, without destroying the spatial relationship of other species in the gel. Accordingly, reading/mapping of an array of species on the gel can be used to direct sequential removal of species of interest, without needing to compensate for gel distortion. The process can thus be effectively automated, e.g. using robotic means for removal or excision of the relevant gel portions, on a reproducible basis.

A second aspect of the invention lies in apparatus for use in analysing species separated on a gel, which comprises:

means for detecting the array of species, e.g. by labelling thereof, on the gel, the gel being fixed to a support that is substantially non-interfering with respect to the detection of a detectable label carried by the species;

selecting one or more points of the array, as containing species for analysis;

removing the gel at each of said one or more points; and

analysing the contents of the removed gel.

A third aspect of the invention is based on the appreciation that a novel, automatable, integrated system has been provided, in which materials from biological systems can be identified without human intervention. A remote-controlled system is made available, for the recovery, identification and characterisation of materials such as proteins and carbohydrates. The process comprises:

(i) separating the species, e.g. before or after labelling them, by applying the contents of the sample to a gel, the gel being fixed to a support that is substantially non-interfering with respect to the detection of the label;

(ii) mapping the resultant array of species;

(iii) selecting a point of the array, as containing species for analysis;

(iv) removing gel at the selected point;

(v) analysing the contents of the removed gel; and

(vi) repeating steps (iii), (iv) and (v), for another point of the array, thereby recovering and characterising individual species.

Description of the Invention

Most components used in the present invention are themselves known. Their use will therefore be familiar to the skilled man, making implementation of the invention quite simple.

The invention is particularly suitable for application to the detection of species carrying a fluorescent label. Such labels are well known. The gel, e.g. a polyacrylamide, may also be conventional.

Especially for use with fluorescent labels, the preferred support is glass. Polyacrylamide gels can be covalently bonded to glass. Such "gels on glass" are (i) compatible with both fluorescent-based, densitometry-based, and spectroscopy-based detection of proteins in gels; (ii) may be covalently attached to the glass during or after polymerisation, and (iii) show unimpaired protein separation properties.

By way of example, the glass or other support may be functionalised, e.g. using a bifunctional linker, and the gel may then be cast onto the functionalised support.

In one practical embodiment, polyacrylamide gels are covalently backed onto glass pre-coated with an appropriate bifunctional agent, one functional element of which covalently attaches to the glass, and the other functional agent to acrylamide. For example, glass plates are coated 3x with a sufficient volume of 0.4% γ-methacryloxy-propyl-trimethoxysilane in ethanol to cover the desired area of glass. Such coated glass plates are then used during casting of polyacrylamide gels. As a result of the

coating, the acrylamide attaches covalently to the glass during polymerisation, yielding polyacrylamide gels covalently attached to the coated glass plate(s).

A supported gel of the invention may comprise gel covalently bonded to one or both faces of a generally planar support such as glass. A supported gel of the invention is then suitable for separating, say, proteinaceous materials, by electrophoresis, in conventional manner.

As indicated above, it is a characteristic of the invention that, once separated, the species are held in a stable array. A portion of the gel containing a single species can now be removed without affecting the spatial integrity of the array. Removal may be by, for example, excision or localised application of an agent that liquifies the gel, so that the desired species can drip out. Removal of portions of the gel containing species that are required for other analysis can be conducted accurately and reproducibly on the basis of the map of labels in the array that can be obtained, if appropriate with reference to a standard. It will be readily appreciated that this is very suitable for computer-controlled analysis and selection.

Excision of the selected gel portions can be conducted using apparatus of the invention. Thus, a robotic device can be provided, under remote software control, using as its reference an image of the protein map obtained following separation of proteinaceous material. This device can be remotely driven to perform excisions/manipulations on a gel in an operator-prescribed and operator-independent manner.

Such a device may comprise (1) a defined "frame" in which the gel is placed for obtaining the reference image, and in which it is retained during all subsequent manipulations; (2) a bed for controlled location of the frame with gel; and (3) a movable x,y coordinate-locating mechanism with drive attached to a changeable

manipulating/excision component which is remotely directed through software for locating gel regions of interest prescribed by an operator and is capable of performing the desired manipulation(s) and delivering gel or gel-derived material to a defined position in a receiving chamber.

If desired, the gel containing separated, labelled species may be used for storing/archiving. This may be before or (since tearing is unlikely) after parts of the gel have been excised. Storage may be at reduced temperature, e.g. as described in British Patent Application No. 9614477.9.

Once excised, known procedures can be used to analyse the content of the gel, i.e. the separated species of interest. Thus, for example, trypsin digestion can be used to break down proteins. The release, labelling and purification of carbohydrates can be conducted in known manner. It will be readily apparent that more than one species can be analysed, in parallel, using apparatus that is integrated with respect to the separated array. Thus, for example, "known" spots can be used to confirm the existence of species that are expected to be present in the sample under investigation, while at the same time obtaining results on other species of interest.

In summary, therefore, the present invention provides an automated process for the recovery of proteins from gels, fragmentation of recovered proteins, recovery of derived fragments and analysis of the fragments to deduce molecular information on the original protein(s) and the associated post-translational modifications. By virtue of the inventions described above, and development of an automated chemistry work-station, all aspects of the described operation are automated, from immediately after a gel image is obtained to completion of molecular analysis of protein elements. This automation allows parallel analysis of multiple protein elements and is operator-independent.

The accompanying drawing is a flow diagram of operations that may be performed on a mixture of different proteinaceous elements, to allow separation, imaging and recovery of individual elements or sub-mixtures of elements, followed their molecular analysis. As has been indicated, all these operations can be provided in an integrated, automated fashion, by virtue of the present invention.

The invention is based on the initial separation of a protein pool, whether from a body fluid, cellular or sub-cellular extract, using 2-D gel electrophoresis. This process remains unquestionably the highest resolution technique for separating large complex mixtures of proteins, being capable of resolving usually ~5000 proteins from a mixture. The technical drawbacks that have previously limited the use of 2-D gels, namely irreproducibility of gel casting, gel distortion leading to difficulties in image analysis, gel archiving, and so on, have been overcome by means of the invention. The 2-D separation hardware and procedures have been redesigned to allow systematic, controlled, and high-throughput generation of reproducible 2-D gel images.

In an illustrative procedure, gel images are analysed using proprietary software to compare samples with respect to unique and common proteins and also for sample comparison with respect to coordinate expression of "clusters" of proteins (to allow analysis of co- and anti-expression). Identified proteins of interest are recovered from the gel(s) using a software-controlled robotic device, the use of which allows automated, operator-independent protein recovery in a 96-well plate format. This robotic device delivers excised proteins to a second robotic chemistry work-station which functions to perform two operations. First, in-gel proteolytic digestion is performed on each protein, the derived peptides and glycopeptides are extracted and separated by micro-HPLC. This is interfaced directly to an electrospray mass

spectrometer allowing totally automated proteolysis-LC-ES-MC/MS on batches of 96 samples. Peptide sequences are searched against protein and DNA databases for correspondence and archived in an expanding database of peptide MS/MS mass and sequence information. The second function of the chemistry work station is to perform glycosylation analysis on each glycoprotein recovered from the gel, using proprietary chemistries and separation technologies.

This entire process is designed to function as a single integrated "cell" capable of rapidly characterising proteins at the low ng level and performing glycosylation analysis on a corresponding number, at the high ng level. The entire process is controlled and driven by a complete LIMS system which both tracks sample information including patient medical data and inputs data from each operation into a corresponding expanding dataset.

A full bioinformatics capability is thus being developed, in which underlying data sets, each consisting of annotated public domain data and proprietary data are able to be cross-interrogated using a variety of algorithms. The underlying datasets range from detailed sample information to gene expression and EST databases through protein expression and sequence databases to a database of post-translational modification. In this way, the full wealth of available biological data is integrated into the sample/disease-specific analysis of protein and post-translational modification, to allow a more systematic and detailed analysis of a particular biological system or process.

The benefits of this technology derive primarily from coupling complex high-resolution "images" of protein expression with a high throughput, automated process for characterising expressed proteins, glycoproteins and post-translational modifications. The bioinformatics software leverages this information using the growing genome and EST databases to couple DNA to protein and post-translational

modification information. This in turn will allow deeper analysis and insight into the biochemical mechanisms underlying a biological process of interest and so to a description of the proteins/glycoproteins central to that process.

Numerous applications of this protein-based technology are envisaged especially in providing the following:

- a deeper understanding of the biochemical basis of several human disease mechanisms and of cellular activation by parallel analysis of sets of proteins;
- discovery of more relevant molecular targets for a given disease process, including the very large (>1000) but untapped family of enzymes involved in post-translational modification, protein folding and intra-cellular trafficking;
- databases of differentially expressed proteins;
- new protein-based diagnostic and surrogate markers for disease;
- characterisation of the serum biochemical phenotypes of patients during clinical studies to correlate response to expressed serum markers;
- characterisation of the biochemical phenotype of fungal and other microbial organisms;
- biochemical analysis of the effects of proposed drugs on individual cell/tissue-types, prior to/during clinical trials.

CLAIMS

1. A supported gel suitable for use in electrophoresis, in which the gel is covalently bonded to a solid support such that the gel has two-dimensional spatial stability, and the support is substantially non-interfering with respect to detection of a detectable label carried by the species.

2. A supported gel according to claim 1, wherein the support is glass.

3. A supported gel according to claim 1 or claim 2, wherein the gel is polyacrylamide.

4. A supported gel according to any preceding claim, wherein the gel and the support are bonded *via* a bifunctional linker.

5. A supported gel according to claim 4, obtainable by functionalising the support, and casting the gel onto the functionalised support.

6. A supported gel according to any preceding claim, wherein the label is fluorescent.

7. A supported gel according to any preceding claim, including a plurality of separated labelled species.

8. A supported gel according to claim 7, wherein the species are proteinaceous.

9. A supported gel according to claim 7 or claim 8, from which one or more portions have been removed, without affecting the spatial stability of the gel.

10. An assay for labelled species by gel electrophoresis, which comprises separating the species on a gel according to any of claims 1 to 6, removing one or more portions of the gel containing separated species, without affecting the spatial stability of the gel, and analysing the species in said one or more portions.

11. Apparatus for use in analysing species separated on a gel, which comprises:

means for detecting the array of species on the gel, the gel being fixed to a support that is substantially non-

interfering with respect to the detection of a detectable label carried by the species;

selecting one or more points of the array, as containing species for analysis;

removing the gel at each of said one or more points; and

analysing the contents of the removed gel.

12. Apparatus according to claim 11, which comprises robotic means for excising discrete portions of the gel.

13. Apparatus according to claim 11 or claim 12, wherein the gel is a supported gel according to any of claims 1 to 8.

14. An automated, integrated process for analysis of species in a biological sample, which comprises:

(i) separating the species by applying the contents of the sample to a gel, the gel being fixed to a support that is substantially non-interfering with respect to the detection of a detectable label carried by the species;

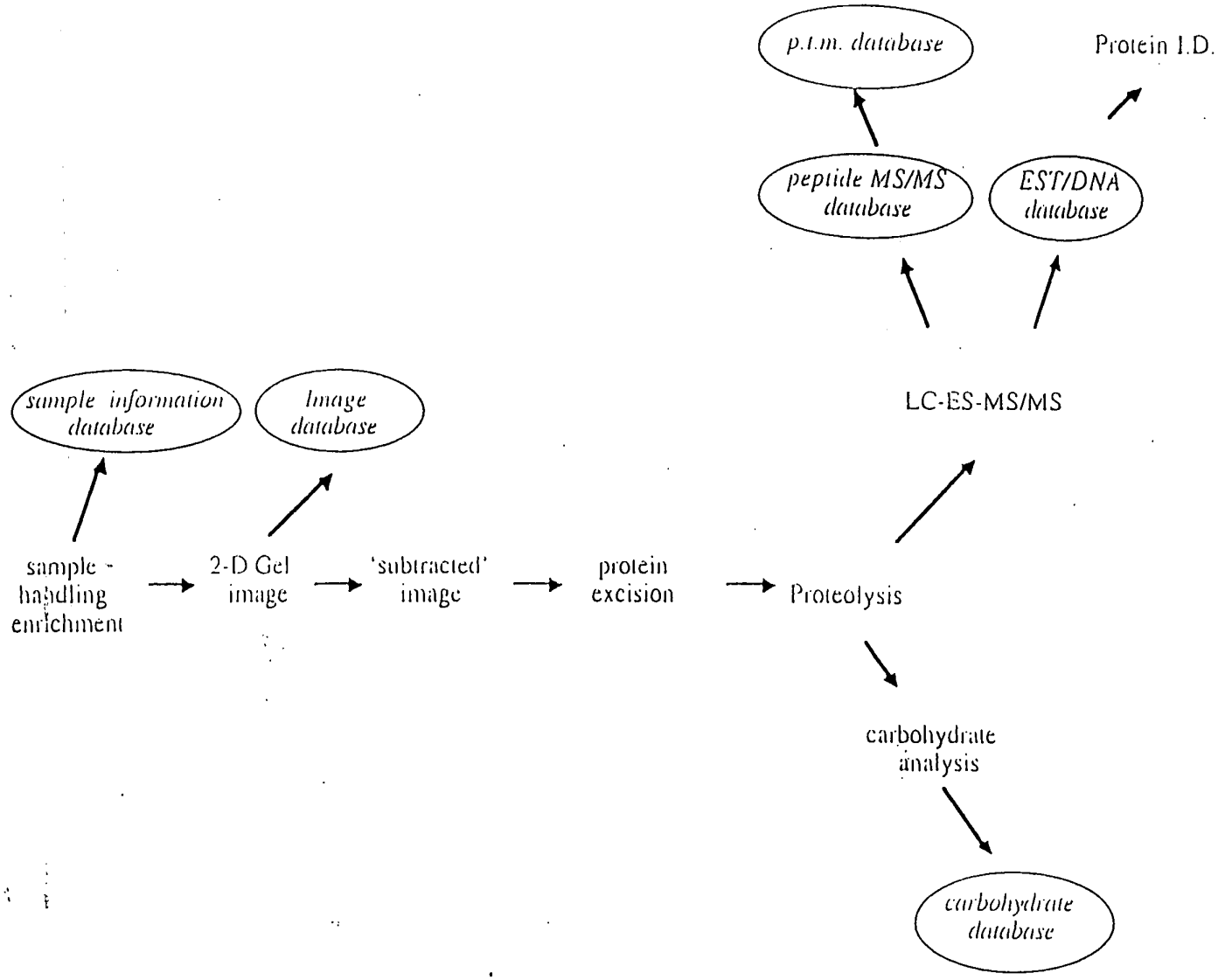
(ii) mapping the resultant array of species;

(iii) selecting a point of the array, as containing species for analysis;

(iv) removing gel at the selected point;

(v) analysing the contents of the removed gel; and

(vi) repeating steps (iii), (iv) and (v), for another point of the array, thereby recovering and characterising individual species.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